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~~RUNNING HEAD: EFFECTS OF MASTITIS ON MILK YIELD~~

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EFFECTS OF CLINICAL MASTITIS ON MILK YIELD IN DAIRY COWS

P. J. RAJALA[*,†1], Y. T. GRÖHN[*], C. E. McCULLOCH[††], and C. L. GUARD[*]

[*] Department of Population Medicine and Diagnostic Sciences,
College of Veterinary Medicine,
Cornell University, Ithaca, NY 14853
[†] Department of Clinical Veterinary Sciences,
Faculty of Veterinary Medicine, P.O. Box 57,
FIN-00014 Helsinki University, Finland
[††]Biometrics Unit,
Department of Statistical Science
Cornell University, Ithaca, NY 14853

[1]Phone number: 607-253-3571
Fax number: 607-253-3083

ABSTRACT

The effect of clinical mastitis on milk yield was studied in 24,276 Finnish Ayrshire cows that calved in 1993 and were followed for one lactation (i.e., until culling or the next calving). Only cows that had mastitis, but no other diseases and cows that had no diseases (healthy cows) during the lactation were included in the study.

Monthly test day milk yields were treated as repeated measurements within an animal in a mixed model analysis. Mastitis index categories were created to relate the timing of mastitis to the test day milk measures. Statistical models (a separate model for each parity) included fixed effects of calving season, stage of lactation and mastitis index. An autoregressive correlation structure was used to model the association among the repeated measurements. Clustering of cows within herds was also accounted for in the model. The effect of mastitis occurring at different periods during the lactation was studied.

The daily loss during the first two weeks after the occurrence of mastitis varied from 1.0 kg to 2.5 kg, and the total loss over the entire lactation varied from 110 kg to 552 kg, depending on parity and the time of mastitis occurrence. Regardless of the time of its occurrence during the lactation, mastitis had a long lasting effect on milk yield: mastitic cows never reached their pre-mastitis milk yield level during the rest of the lactation after the disease onset.

Key words: mastitis, milk yield, repeated measures, mixed model analysis

INTRODUCTION

Mastitis is one of the most common dairy cow diseases (3, 6, 9, 17, 20) and it can cause considerable losses to dairy farmers. The losses accrue from several sources (19), one of which is decreased milk yield. Several studies have found that clinical mastitis has a detrimental effect on milk yield (1, 5, 12, 16). Subclinical mastitis or high somatic cell count (SCC) has also been associated with decreased milk yield (8, 10). The carry-over effect of mastitis and high SCC from one lactation to the next has been found to be, in general, statistically significant but small (10), and only if the cow had 3 or more infected quarters was her yield affected in the next lactation (12). The effect of clinical mastitis can be different depending on at what stage of lactation the disease occurs (15). Results stating a beneficial effect of clinical diseases of the udder on milk yield have also been reported (8); in that study, the effect was attributed to the therapy provided to the cows affected with the condition.

One general problem in much previous research on the effect of diseases on milk yield is that the focus has been on the entire 305-d lactation curve. 305-d milk yield can not capture short term fluctuations and decreases in milk yield. Cows with mastitis are often higher-yielding cows and they produce more, even having contracted the disease, than their healthy, in general lower-yielding herdmates (11). So, when using a summary measure, like 305-d milk yield, erroneous conclusions are possible due to faulty assumptions and choice of inadequate statistical methods. More recently, approaches considering monthly or daily milk measurements have been advocated.

The purpose of this study was to estimate the effect of clinical mastitis on milk yield in Finnish Ayrshire cows using monthly test day milk yields.

MATERIALS METHODS

Data

The data for this study were from 24,276 Finnish Ayrshire dairy cows that calved during 1993 and were followed until the next calving or culling. The cows were in herds that belonged to the milk registry and the national dairy cow health recording system. These data are a subset of a larger study population of 39,727 Finnish Ayrshire cows, which have been described in detail previously (17). Only cows that had no diseases and cows that had only mastitis, but no other diseases during the study lactation, were included in the current study.

Finnish farmers do not have access to veterinary drugs without supervision of a veterinarian so virtually all diseases are diagnosed and treated by a veterinarian during farm visits. The veterinary diagnosis of clinical mastitis was used for this study. Diagnoses were made according to ordinary clinical methods under normal field conditions. Only the first occurrence of mastitis was considered in this study. Calving dates, disease dates and dates for monthly test day milk sampling were available.

Monthly test day milk yields, taken at approximately 30 d intervals, were used to study the effect of mastitis on milk yield. The lactation was divided into 17 stages: milk records taken within 60 d after calving were grouped by 10-d intervals, records from 61 to 180 d were grouped into 20-d intervals and records from 181 d on formed 30-d intervals. Only test day milk yields until 330 d after calving were considered.

Parity had four levels: 1, 2, 3, and 4 or higher. Four calving seasons were defined by 3-month intervals: winter, December to February; spring, March to May; summer, June to August; and fall, September to November.

Statistical analysis

In these data, repeated measurements were present in both space and time. Cows within the same herd were clustered in space and repeated measurements of daily milk yields of the same cow were correlated in time. What makes the repeated measures data analysis distinct from simple linear models is the covariance structure of the observed data. In a typical repeated measures experiment, two measurements taken at adjacent times are typically more highly correlated than two measurements taken several time points apart (14).

One type of statistical analysis that can be used for repeated measures is based on the mixed model with a special parametric structure for the covariance matrices. This type of methodology has been computationally feasible only in recent years. It is applied in PROC MIXED of SAS, typically using the REPEATED statement (14). This procedure was used for these data with the monthly test day milk yields as the outcome variable. A cow will usually have approximately 10 monthly test day milk yields recorded during a lactation. Because milk yield measurements from the same lactation for a cow are correlated, it is important to account for this correlation in estimating the effects of disease on milk yield.

In our previous study (18) we compared three commonly used correlation structures (simple, compound symmetry and first-order autoregressive) and found the first-order autoregressive correlation structure to provide the best fit to these data.

In PROC MIXED, the standard linear model is generalized to form a mixed model: $y = X\beta + Z\gamma + \epsilon$ with $Var(\gamma) = G$ and $Var(\epsilon) = R$, so that $Var(y)=ZGZ' + R$, where **y** = vector of test day milk yields, β = vector of fixed effects, γ = random herd effects and ϵ = vector of random errors.

A correlation pattern can be modeled in PROC MIXED in two ways, either by introducing a correlation pattern in the random effects, γ through a nonidentity matrix G or by an R matrix so that it equals σ^2 multiplied by some nonidentity matrix.

The effects of mastitis on test day milk yields were studied separately for each parity (i.e., parities 1, 2, 3, and 4 or higher). Calving season, stage of lactation, and disease variables were fixed effects in each model. Clustering of cows within herds was accounted for by indicating in the model, in the REPEATED statement, that cows were nested within herds.

Analysis 1. In this analysis the yield of healthy cows was used as the comparison level. To differentiate between cows with and without mastitis, a disease index variable was created for each test day milk yield in order to study the effects of mastitis on milk yield.

The mastitis index variable was defined as follows: 1 for test day milk yields collected more than 28 days before the diagnosis, 2 for test day yields collected between 15 and 28 d before the diagnosis, 3 for test day milk yields collected within 14 d before the diagnosis, 4 for test day yields collected within 14 days after the diagnosis, 5 for test day yields collected between 15 and 28 days after the diagnosis, 6 for test day yields collected between 29 and 42 days after the diagnosis, 7 for test day yields collected later than 42 days after the diagnosis, and 8 if the cow had not been diagnosed with mastitis, i.e., the milk yield of the healthy cows was considered as the reference level.

Analysis 2. In these analyses the milk yield level of the mastitic cows more than four weeks prior to the clinical onset and diagnosis of the disease was used as the reference level. To study whether mastitis had a different effect depending on the stage of lactation when it occurs, three periods for mastitis occurrence were considered: 1) before the peak yield (period 1); 2) between the peak and 120 d after calving (period 2); or 3) later than 120 d after calving (period 3). The peak for each parity was calculated using Wood's equation $y_t = at^be^{-ct}$ (21), where y_t = test day milk yield on day t and the peak occurs (b/c) days after calving.

For period 1, the mastitis index was defined as in analysis 1 except for the following differences: 1 if the cow was healthy (i.e., had not been diagnosed with mastitis), 2 for test day yields collected between 7 and 14 days before the diagnosis (i.e., 1-wk period), 3 for test day milk yields collected within 7 d before the diagnosis (i.e., 1-wk period), and 8 for test day milk yields collected more than 28 d before the mastitis diagnosis from cows that had mastitis after the peak (this was the reference level).

For periods 2 and 3 the mastitis index was defined as previously with the following changes: 2 for test day yields collected between 15 and 28 d before the diagnosis (i.e., 2-wk period), 3 for test day milk yields collected within 14 days before the diagnosis (i.e., 2-wk period), and 8 for test day milk yields collected more than 28 d before the diagnosis (the reference level).

RESULTS AND DISCUSSION

Table 1 presents the lactational incidence risks (LIR) for clinical mastitis by parity in these data (i.e., healthy cows and cows with only mastitis, later also referred to as the "mastitis data") and in the entire data set from which this subset of data came from (17). Lactational incidence risk was calculated by dividing the number of cows with at least one episode of mastitis by the total number of cows at risk and multiplied by 100 (as it was presented as a percentage). The overall LIR for mastitis in the mastitis data was 14.0 %; in parities 1, 2, 3, and 4 or higher it was 12.1 %, 14.3 %, 14.9 % and 15.9 %, respectively. In the entire data set the corresponding lactational incidence risks were 17.0, 14.2, 16.5, 17.6 and 20.2. This shows that the LIR's were lower in the mastitis data, suggesting that cows often have some other diseases besides mastitis during the lactation. This seems to hold true especially for the older cows.

We restricted our analysis only to cows that had no diseases at all (referred to as healthy cows) and to cows that had only mastitis, but no other diseases during the study lactation. Therefore, we were able to ensure that the estimates for the mastitis effect on milk yield were not confounded by any other diseases, but that the effect could truly be attributed to mastitis and mastitis only.

The peak of the yield in these data for parity 1 cows occurred on day 58, for parity 2 cows on day 39 and for cows in parity 3 and 4 or higher the peak occurred on day 40.

Analysis 1. The results from Analysis 1, which compared the milk yield of mastitic cows to that of the healthy cows, clearly indicated that before contracting the disease mastitic cows produced more milk than the healthy cows (Figures 1 and 2). The bars in Figure 1 represent differences in daily milk yields between cows with mastitis and healthy cows. A zero value indicates equal production between healthy cows and cows with mastitis, positive values indicate that cows with mastitis are out-yielding healthy cows. Milk yield began to decline four weeks before the clinical onset of mastitis in all parities and dropped below the yield level of the healthy cows during the first two weeks after the diagnosis. Yield started to increase after this, but it never reached the level it was at more than four weeks before the mastitis onset (Figure 1).

Figure 2 shows the lactation curves of healthy and cows with mastitis in parity 2. The lactation curves in all the other parities followed the same pattern. It is apparent from the figure that cows with mastitis produced more than their healthy counterparts, despite the disease. This is in accordance with our previous study (17), which showed that increasing milk yield was a risk factor for mastitis in

Finnish Ayrshire cows. Other studies have also reported increasing mastitis risk with increasing milk yield (2, 11).

Mastitis clearly affected the milk yield, but the difference between the milk yield of the healthy and the mastitic cows after mastitis was not statistically significant; mastitis merely brought the yield of the cows that contracted the disease to the same level at which the healthy cows were. Thus, these results suggested that comparing the yield of mastitic cows to that of the healthy cows was not the most appropriate approach to the problem of estimating the effect of mastitis on milk yield. One needs to calculate the loss indirectly from the pre-mastitis yield level, otherwise the true effect of mastitis would be drastically underestimated. Therefore, the milk level of cows with mastitis prior to mastitis onset was chosen as the reference point for further analyses on modeling the mastitis effect on milk yield.

Analysis 2. Tables 2 to 4 present the results from Analysis 2, each table presenting results for one of the lactation periods. In these analyses the milk yield level of cows with mastitis more than four weeks prior to the clinical onset of the disease was used as the reference category.

When mastitis occurred during early lactation (before the peak), the daily losses during the first two weeks after the clinical onset of the disease varied from 1.1 kg to 2.5 kg, depending on parity (Table 2). The yield never reached the pre-mastitis level for the rest of the lactation in any parity. The total loss due to mastitis during the lactation varied between 294.1 and 551.8 kg (the total loss was calculated assuming a 305-d lactation and mastitis occurring on day 7). The amount of milk lost increased with increasing parity, indicating that higher producing, older cows lost more. The loss in parity 1 cows was 4.6% of the overall lactational 305-d yield, in parities 2, 3 and 4 or higher it was 4.1%, 6.9 % and 7.4%, respectively.

The problem in estimating the effect of mastitis in the early stage of lactation on milk yield was that a large proportion of the cows had mastitis so early that they did not have any "healthy" milk measures taken before the disease. Therefore, we used the pre-mastitis (more than four weeks before the onset) milk yield of those cows that contracted mastitis later during the lactation as the reference level.

When mastitis occurred between peak and 120 d, the daily losses within the first two weeks after the diagnosis of mastitis varied between 1.3 kg and 2.1 kg (Table 3). There was no significant milk reducing effect due to mastitis prior to the clinical onset of the disease. The overall losses due to mastitis occurring between peak and 120 d varied between 300 kg and 352 kg (the loss was calculated assuming a 305-d lactation and mastitis occurring on day 90). The youngest cows (parity 1) seemed to be affected most severely (proportionally) by mastitis occurring in this period. Cows with mastitis never reached their pre-mastitis yield level, but remained at a significantly lower level for the rest of the lactation in all parities.

When mastitis occurred during the late lactation, the milk reducing effect was apparent two to four weeks prior to the clinical disease, suggesting the presence of subclinical mastitis (Table 4). This is in agreement with the results of Deluyker (7), Dohoo and Martin (8), and Fetrow et al (10); they showed that subclinical mastitis was associated with decreased milk yield. The cows never totally recovered from the disease, but milked between 0.7 and 2.5 kg less per day (depending on parity) for the rest of the lactation than they would have without mastitis.

Lucey and Rowlands (15) reported that mastitis can have a different effect depending on the stage of lactation in which it occurs. They found the reduction in 305-d yield to be greatest when clinical mastitis occurred before the peak. Also, Lescourret and Coulon (13) reported that the impact of mastitis appeared to be more marked in early than late lactation. These are in agreement with our results in general which also suggest that the losses due to mastitis were greatest when mastitis occurred in early lactation (before the peak). Yield of the oldest cows seemed to be most affected when mastitis occurred before the peak, whereas the youngest cows (parity 1) lost most milk due to mastitis between the peak and day 120.

In general, we found that milk yield was significantly affected by mastitis; the reduction in 305-d yield was estimated to vary between 1.8% and 7.4 %. However, it is still possible that our estimates underestimate the true effect. If a cow had a severe case of mastitis on a test day, it is possible that no milk measures were taken from her on that day. Also, she might have been culled early in lactation due to mastitis before any milk measures were taken. Both of these scenarios would cause underestimation of the real loss.

Deluyker et al. (7) estimated that occurrence of clinical mastitis was associated with 5% milk yield loss , which is in agreement with our estimates. Several other studies have also found clinical mastitis to have a detrimental effect on milk yield (1, 5, 12, 16). When mastitis occurred during late lactation, the yield had already started to decline two to four weeks prior to the clinical onset of mastitis; the greatest reduction, however, was seen right after the diagnosis. After the cow had contracted mastitis, her milk yield never returned to the pre-mastitis level, but remained significantly lower throughout the rest of the lactation. Also, Lescourret and Coulon (13) reported that in more than 1/3 of the cases of mastitis milk yield was affected for an extended period. Bunch et al. (4) even suggested that once a cow has contracted mastitis it is unlikely to achieve its full milk-yield potential in the next lactation.

The results from all of these analyses clearly showed that mastitic cows produced more than their healthy counterparts. The daily yield of the healthy cows was, on average, 0.7 to 1.9 kg less than the pre-mastitis yield level of the cows that contracted mastitis at some point of the lactation (Tables 2-4). Therefore it is of great importance to carefully consider the reference level used when interpreting results from an analysis estimating the effects of mastitis on milk yield. Directly comparing the yield of mastitic cows to that of healthy cows and interpreting that as a loss due to mastitis would most likely underestimate the effects of the disease.

One of the strengths of this study was the comprehensive data base with veterinary diagnosed diseases. Due to the large data set we were able to include in the study only cows with no diseases at all and cows with only mastitis and no other diseases. The effects of mastitis on milk yield were thus not confounded by any other diseases. Also, a mixed model analysis with repeated measurements is the most sophisticated and accurate method currently available to measure milk loss due to diseases. It allows researchers to detect short-term effects and also to estimate milk losses both before and after clinical mastitis. A novel approach to estimate the milk loss, when mastitis occurs early in the lactation and cows do not have any non-affected milk measures taken before the disease, was to use the pre-mastitis milk yield of cows contracting mastitis later during the lactation as the comparison level.

CONCLUSIONS

The daily losses due to clinical mastitis varied between 1.0 kg and 2.5 kg during the first two weeks after the diagnosis of the disease, and the overall loss due to mastitis over the lactation varied between 110 kg and 552 kg, depending on parity and the time of mastitis occurrence. When mastitis occurred during late lactation, the decline of milk yield started two to four weeks prior to the onset of the clinical mastitis, suggesting the presence of subclinical mastitis. Mastitis has a long lasting effect on milk yield; after contracting mastitis a cow was not able to reach her pre-mastitis milk yield level again during the rest of the lactation.

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Table 1. Lactational incidence risks[1] (LIR, %) of clinical mastitis by parity in Finnish Ayrshire cows.

	Parity 1	Parity 2	Parity 3	Parity 4+	Overall
Entire data set[2]	14.2	16.5	17.6	20.2	17.0
Mastitis data set[3]	12.1	14.3	14.9	15.9	14.0

[1] (No. of cows with mastitis / no. of cows at risk) * 100%
[2] 39, 727 Finnish Ayrshire cows that calved in 1993 and were followed for one lactation.
[3] A subset of the above mentioned data set; includes only cows that had no diseases and that had mastitis and no other diseases during the lactation, consisted of 24,274 cows.

Table 2. Effects of mastitis occurring before the peak yield on milk yield (kg) in 24,276 Finnish Ayrshire cows that calved in 1993 and were followed for one lactation[1].

	Parity 1		Parity 2		Parity 3		Parity 4+	
Effect [2]	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
14-8 d BD	-1.5*	0.5	-1.0	1.0	-0.1	1.2	-1.9	1.0
7-1 d BD	-1.0	0.6	-0.8	0.7	-2.4**	0.9	-2.2**	0.7
0-14 d AD	-1.7***	0.3	-1.1**	0.4	-2.5***	0.5	-1.8***	0.5
15-28 d AD	-1.2***	0.3	-0.7	0.4	-2.3***	0.5	-2.0***	0.4
29-42 d AD	-0.9***	0.3	-0.9*	0.4	-1.1**	0.5	-1.6***	0.4
> 42 d AD	-0.9***	0.2	-1.0***	0.3	-1.6***	0.4	-1.8***	0.3
Healthy cow	-0.9***	0.2	-1.3***	0.2	-1.6***	0.3	-1.6***	0.3
Total loss[3]	-294.1	(4.7%)[4]	-284.0	(4.1%)[4]	-509.0	(6.9%)[4]	-551.8	(7.4%)[4]

[1] The reference level is the milk yield prior to mastitis onset of cows that contracted mastitis after the peak. Calving season and stage of lactation were included in the model as fixed effects.

[2] Period when the test day milk sample was collected with respect to the diagnosis of clinical mastitis (BD = before diagnosis, AD = after diagnosis).

[3] The total loss was calculated assuming a 305-d lactation and mastitis occurring on day 7 after calving.

[4] Percentage loss, calculated from the overall lactational 305-d production

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

Table 3. Effect of mastitis occurring between the peak and day 120 on milk yield (kg) in 24,276 Finnish Ayrshire cows that calved in 1993 and were followed for one lactation[1].

	Parity 1		Parity 2		Parity 3		Parity 4+	
	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
28-15 d BD	-0.8*	0.4	-0.3	0.4	-0.3	0.4	0.6	0.4
14-1 d BD	-0.7	0.4	-0.3	0.4	-0.8	0.4	-0.3	0.4
0-14 d AD	-1.5***	0.4	-1.7***	0.4	-2.1***	0.5	-1.3***	0.4
15-28 d AD	-1.4**	0.5	-1.2**	0.4	-1.9***	0.5	-1.3**	0.4
29-42 d AD	-1.4**	0.5	-1.2**	0.4	-1.4**	0.5	-1.1**	0.4
> 42 d AD	-1.6**	0.4	-1.4***	0.4	-1.6***	0.5	-1.6***	0.4
Healthy cow	-0.7*	0.4	-1.3***	0.4	-1.5	0.4	-1.6***	0.4
Total loss[3]	-348.2	(5.6%)[4]	-299.6	(4.3 %)[4]	-352.4	(4.8%)[4]	-328.6	(4.4%)[4]

[1] The comparison is to the cow's own milk yield level more than four weeks prior to the clinical onset of the disease. Calving season and stage of lactation were included in each model as fixed effects.

[2] Period when the test day milk sample was collected with respect to the diagnosis of mastitis (BD = before diagnosis, AD = after diagnosis).

[3] The total loss was calculated assuming a 305-d lactation and mastitis occurring on day 90 after calving.

[4] Percentage loss, calculated from the overall lactational 305-d production

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

Table 4. Effect of mastitis occurring after day 120 on milk yield (kg) in 24,276 Finnish Ayrshire cows that calved in 1993 and were followed for one lactation[1].

	Parity 1		Parity 2		Parity 3		Parity 4+	
Effect[2]	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
28-15 d BD	-0.4	0.2	-0.8***	0.3	-0.9***	0.3	-0.2	0.3
14-1 d BD	-0.5*	0.2	-1.1***	0.2	-1.3***	0.3	-0.7*	0.3
0-14 d AD	-1.0***	0.3	-1.8***	0.3	-2.4***	0.4	-1.8***	0.4
15-28 d AD	-0.5	0.3	-0.8**	0.3	-2.1***	0.4	-1.4***	0.4
29-42 d AD	-0.7*	0.3	-1.5***	0.4	-2.4***	0.5	-1.4***	0.5
> 42 d AD	-0.7*	0.3	-1.2***	0.3	-2.3***	0.4	-2.5***	0.4
Healthy cow	-0.9***	0.2	-1.4***	0.2	-1.9***	0.3	-1.6***	0.3
Total loss[3]	-109.9	(1.8%)[4]	-219.6	(3.1%)[4]	-387.3	(5.2%)[4]	-356.7	(4.8%)[4]

[1] The comparison is to the cow's own pre-mastitis milk yield level more than four weeks before the clinical onset of the disease. Calving season and stage of lactation were included in each model as fixed effects.

[2] Period when the test day milk sample was collected with respect to the diagnosis of clinical mastitis (BD = before diagnosis, AD = after diagnosis).

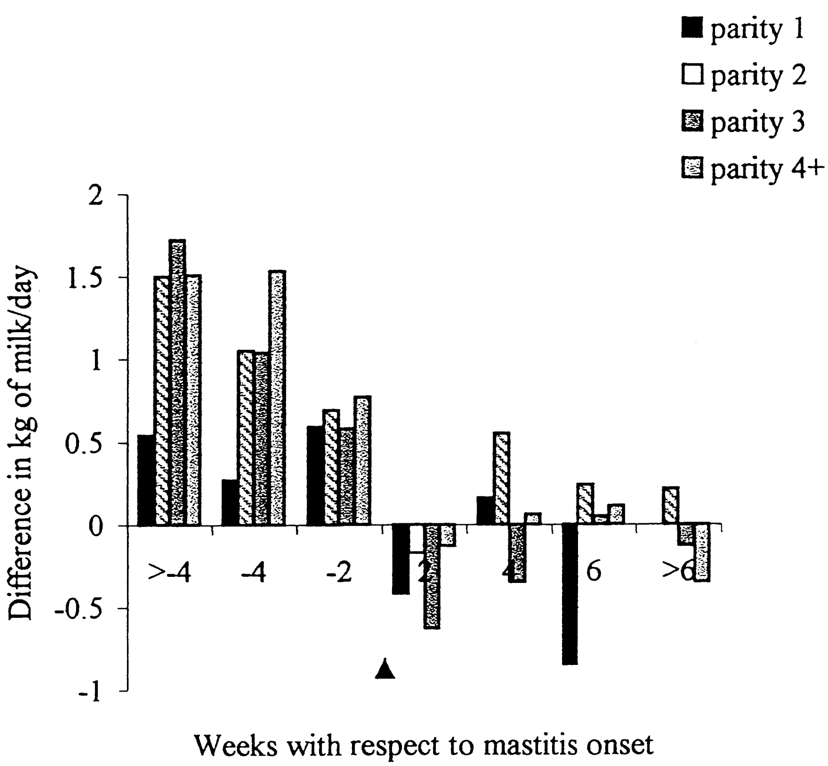
[3] The total loss was calculated assuming a 305-d lactation and mastitis occurring on day 150 after calving.

[4] Percentage loss, calculated from the overall lactational 305-d production

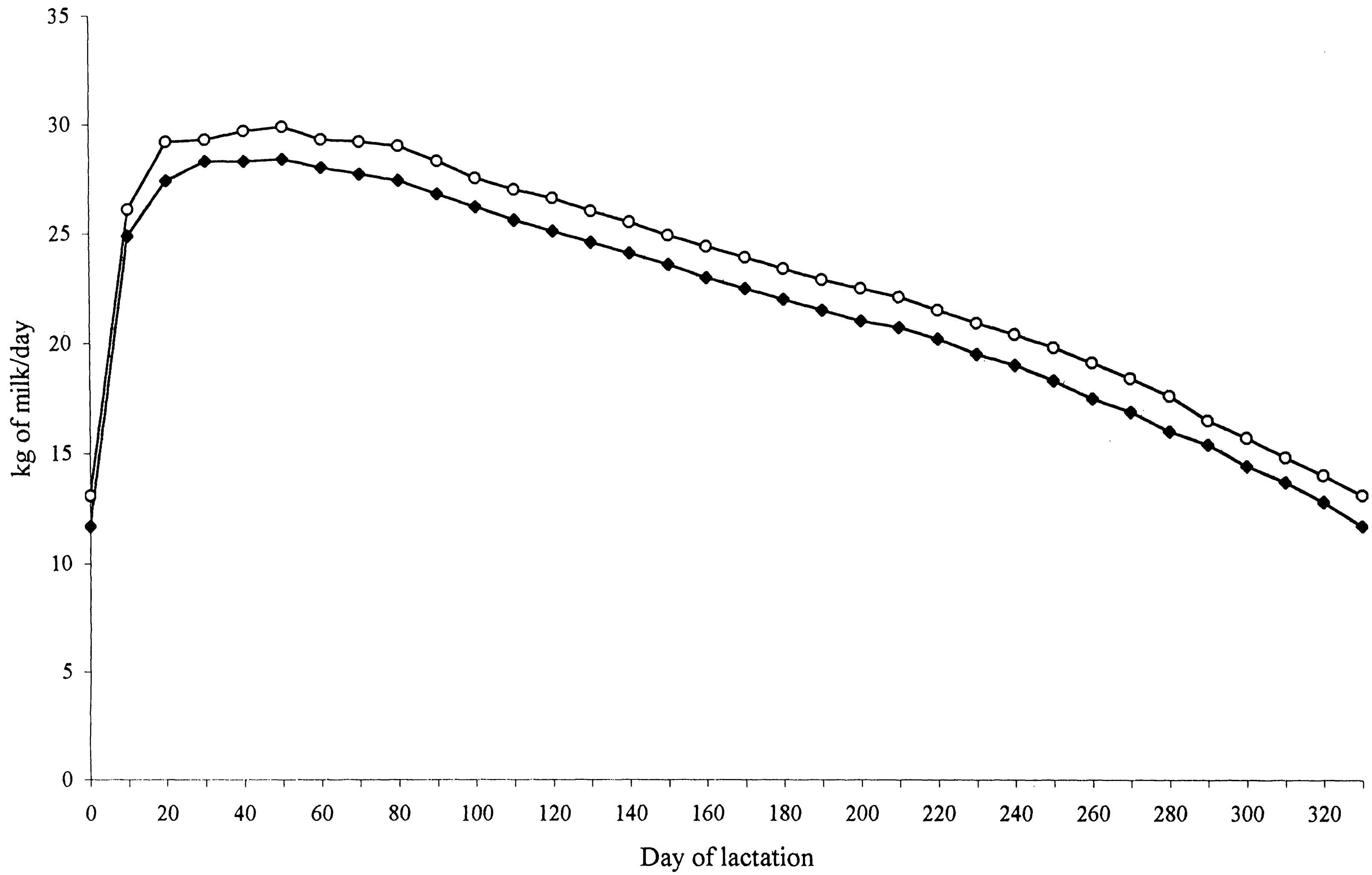
* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

Figure 1. Effect of mastitis on milk yield (kg). Bars represent the daily milk yields of cows with mastitis with healthy cows' milk yield subtracted for reference. Positive values indicate that cows which had mastitis are out-yielding healthy cows. A zero value indicates equal production between healthy cows and cows with mastitis. The little arrow shows the time of mastitis onset.

Figure 2. Lactation curves of healthy cows and cows with mastitis in parity 2.
-♦- healthy cows, -○- cows with mastitis



parity 1
parity 2
parity 3
parity 4+
2
1.5
1
0.5
0
-0.5
-1
Difference in kg of milk/day
>-4
-4
-2
2
4
6
>6
Weeks with respect to mastitis onset


35
30
25
20
15
10
5
0
kg of milk/day
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
Day of lactation